Sunday-244

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A Rapid Single Molecule Counting Method Sensitively Detects Clostridium difficile Toxin B Directly in Stool Samples

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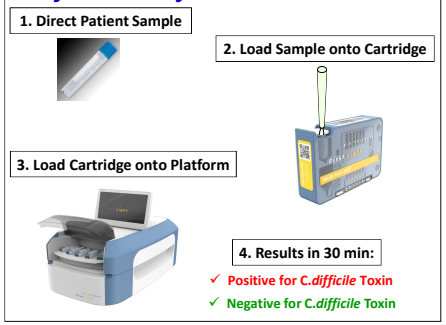
Background

We developed an ultrasensitive MultiPath *Clostridium difficile* toxin B test based on a novel digital imaging technology that counts single target molecules in stool samples with little or no sample preparation. Current tests for *C. difficile* gastrointestinal infection can be inaccurate. *C. difficile* toxin immunoassays often lack clinical sensivity. Nucleic acid amplification tests have excellent clinical sensivity but can have diminished clinical specificity due to their inability to distinguish patients with *C. difficile* infection from patients that are carriers of *C. difficile* organisms. Because production of toxin is a hallmark of *C. difficile* infection, an ultrasensitive *C. difficile* toxin test, such as the one presented in this report, could address the issues associated with the current tests and offer improved accuracy for detecting patients with this devasting infection.

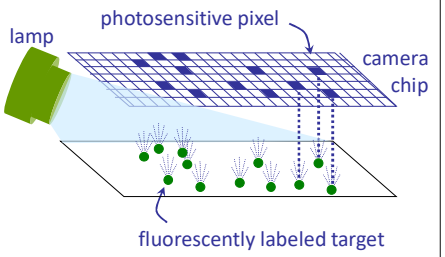
Technical Approach

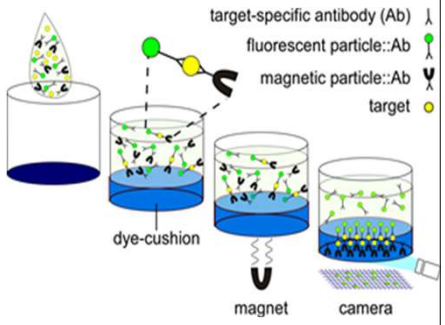
- The MultiPath *C. difficile* toxin B test uses non-magnified digital imaging to count target-specific magnetic and fluorescent particles that have been tethered together by toxin molecules.
- The use of a novel dye-cushion eliminates the need for sample preparation and wash steps.
- Clinical stool samples to estimate the limit of detection, imprecision, and dynamic range.

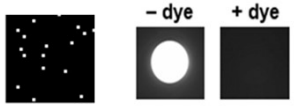
Platform Workflow



Technology

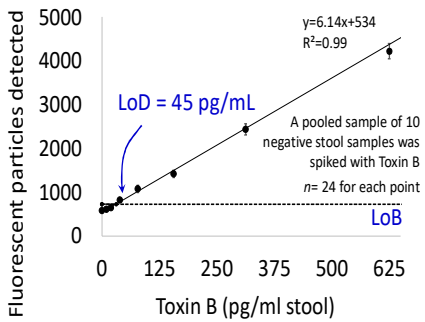


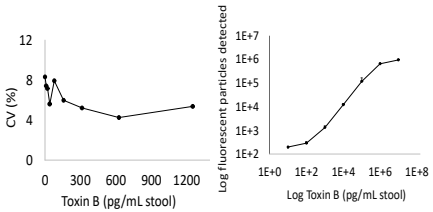




- ✓ Dye-cushion eliminates sample prep and washes
- ✓ 30 minute test turnaround
- ✓ Positive and neutralization internal controls
- ✓ All steps in cartridge on automated analyzer
- ✓ All reagents stabilized in cartridge

Analytical Results





Interference. 20 potentially interfering substances commonly associated with diarrheal stool samples were found to have no impact on the assay results for samples spiked with toxin B.

Inclusivity. Analysis of toxins from strains representing common ribotypes (027, 106, 014, 002, 017, 001, 078, 036, 087) showed similar dose/responses when spiked into a pooled stool sample.

Exclusivity/cross-reactivity. We evaluated the toxin assay performance in stool samples in the presence of 23 commonly encountered off-target species ≥ 1e8 CFU/mL. None of them inhibited detection of toxin B spiked or caused false positive results.

Clinical Feasibility Results

Semi-automated analysis. We used a training set of 320 clinical unformed stool samples from patients suspected of having *C. difficile* infection to select parameters to yield optimum accuracy relative to the cellular cytotoxicity neutralization assay (CCNA) reference test. The assays were conducted using microtiter plates and manual pipetting steps. We compared the results of a commercial enzyme immunoassay and a PCR test to the CCNA results.

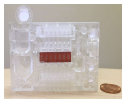
		Reference (CCNA) 1-3 days			
		Positive	Negative		
MultiPath 30 minutes	Positive	96	9	105	Sensitivity 97.0%
	Negative	3	528	531	Specificity 98.3%
		99	537	636	

		Positive	Negative		
Nucleic Acid Amplification	Positive	97	77	174	Sensitivity 98.0%
	Negative	2	460	462	Specificity 85.7%
		99	537	636	

		Positive	Negative		
Enzyme Immunoassay	Positive	21	5	26	Sensitivity 77.8%
	Negative	6	84	90	Specificity 94.4%
		27	89	116	

Fully automated analysis. We tested a random subset of samples on an automated MultiPath Analzer prototype and MultiPath consumable cartridge and compared the results to the CCNA results.

		Positive	Negative		
Multipath Automated Analyzer & Cartridge	Positive	54	10	64	Sensitivity 95%
	Negative	3	304	307	Specificity 97%
		57	314	371	



Limitations. The test detects only *C. difficile* toxin B but not toxin A or binary toxin. The study was not blinded and it treated the samples as a training set to optimize parameters. We only tested unformed stool samples, they had no associated patient information for sub-analyses, and they were not fresh but rather had been frozen at -80°C.

Conclusion

The data presented demonstrate the potential of the ultrasensitive MultiPath technology to deliver rapid, accurate, easy-to-use test for *C. difficile* toxin B. The technology should also have value for a variety of other important infectious disease applications.